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Preview

Stop that Noise and Turn Up the Antisense Transcription

Jan Steensels[1] and Kevin J. Verstrepen[1,*]
[1]VIB Laboratory for Systems Biology and KU Leuven Laboratory for Genetics and Genomics, Centre of Microbial and Plant Genetics, KU Leuven, Kasteelpark Arenberg 22, 3001 Leuven, Belgium
*Correspondence: Kevin.Verstrepen@biw.vib-kuleuven.be
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Many genes are not only transcribed in the sense direction but also yield antisense transcripts. In this issue of *Cell Reports*, Huber et al. (2016) report that some of these transcripts may serve to suppress sense transcription and noise.

The regulatory function of RNA, broadly called RNA interference or RNAi, is best known as a handy tool for reducing the expression of a target gene. Small RNA molecules that show sequence similarity to a specific target gene can block expression by interfering with RNA stability and translation efficiency, explaining the name small interfering RNA (Hammond et al., 2001).

Interestingly however, the first reports of the regulatory role of RNA did not involve short RNAs but rather full-length transcripts. In an attempt to generate more colorful petunias, a Dutch research team introduced extra copies of a flavonoid gene only to find that this resulted in silencing of the gene and pale instead of dark flowers (van der Krol et al., 1990). In the following years, plant researchers increasingly used the introduction of extra copies of a target gene to repress its expression. The exogenous copies were often expressed from the 3′ end of the reading frame, as it was observed that such antisense expression resulted in more efficient suppression. In fact, it is now believed that the original observations in petunia flowers may depend on a small number of antisense transcripts that are formed in cells containing extra gene copies. Artificial antisense-forming constructs were able to repress sense expression in other organisms too, suggesting an evolutionary conserved mechanism.

More recently, studies with tiling arrays or deep sequencing found that antisense transcription also occurs in wild-type cells. For many genes, transcription starts at various sites in the promoter, open reading frame, and terminator regions, and some of these transcripts run in the antisense direction (Bertone et al., 2004; David et al., 2006). Moreover, a key study into the regulation of the initiator of meiosis gene *IME4* in *Saccharomyces cerevisiae* found that antisense expression helps to prevent undesirable *IME4* expression in haploid cells (Hongay et al., 2006). Similarly, antisense transcription also prevents leaky expression in the *S. cerevisiae* galactose network (Lenstra et al., 2015).

Despite the mounting evidence that antisense transcription is quite common and that some long antisense transcripts influence sense transcription, it was still unclear how many of the observed antisense transcripts really affect transcription in the sense direction (Pelechano and Steinmetz, 2013). Moreover, given that *S. cerevisiae* lacks the typical RNAi machinery (Drinnenberg et al., 2011), the mechanism underlying antisense regulation most likely differs from that of typical RNAi. In a study published in this issue of *Cell Reports*, Huber et al. (2016) use an elegant approach to study the genome-wide prevalence, impact, and mechanism of antisense transcription in *S. cerevisiae*. In a technical tour de force, the team used seamless genome-editing techniques to insert the unidirectional *PHO5* terminator element to block the transcription of 162 of the 600 reported *S. cerevisiae* antisense transcripts. Because each of the genes also received a fluorescent tag, it was possible to measure not only the mean population-level effect of blocking antisense transcription at the protein level but also the effect on cell-to-cell variation ("transcriptional noise").

Reassuringly, the results of this new study confirm established cases where antisense RNA has been reported to interfere with sense gene regulation, and the study also detected some new targets. However, depending on the growth environment, blocking the antisense transcript did not markedly affect sense transcription in as many as 75%–88% of genes for which antisense transcripts were detected. In general, genes for which the antisense transcript overlaps the sense transcription start site were affected by antisense transcription, with a higher ratio of antisense-to-sense abundance resulting in stronger effects. In this subset of responsive genes, blocking the antisense transcript results in around 2-fold higher protein levels and an increase in cell-to-cell variation. Together, these results suggest that antisense transcripts might serve to reduce noise and leaky expression of particular genes that require stringent and uniform regulation. Moreover, the results also yield insight into features that may be used to distinguish functional from non-functional antisense transcripts. Specifically, genes for which the antisense transcript overlaps the sense promoter were more likely to be regulated by antisense transcription, suggesting that antisense transcription of the sense promoter may play a role (Figure 1). However, overlap with the sense promoter did not always lead to a significant reduction in sense transcription, indicating that other factors also play a role. Interestingly, antisense-regulated genes show increased H3K4 di- and trimethylation at the 3′ ends of the genes. This histone mark is typically associated with active promoters, but

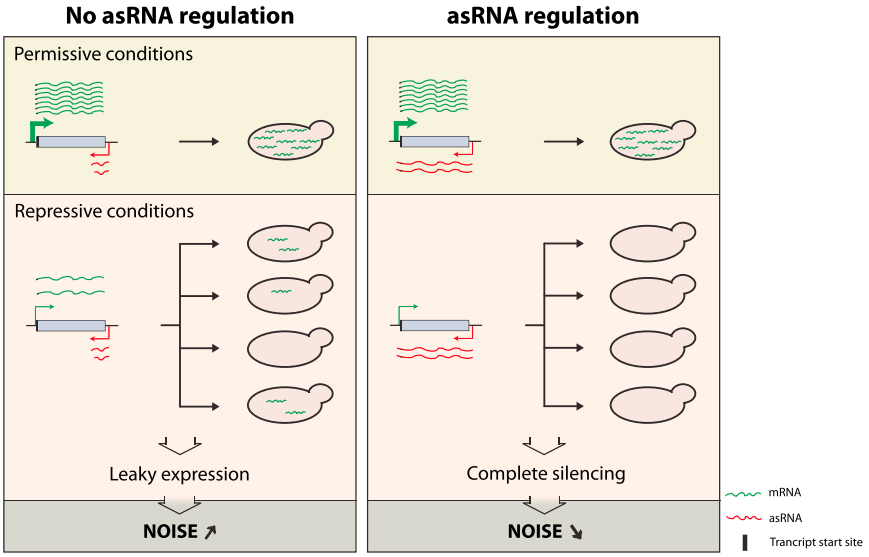

Figure 1. Antisense RNA Regulation Can Reduce Leaky Gene Expression in *Saccharomyces cerevisiae*

Huber et al. (2016) show that antisense regulation correlates with reduced leaky expression of genes in specific conditions, especially when transcripts overlap the sense transcript start site. This not only results in more effective gene silencing but also can reduce heterogeneity in transcript levels between cells ("noise").

antisense transcription might shift it downstream, thereby reducing sense expression.

Why is it that not all antisense transcripts affect sense transcription? First, technical issues might obscure the effect of some antisense transcripts. Insertion of the *PHO5* terminator sequence at the 3′ end of genes, as well as the insertion of a fluorescent tag, might interfere with the desired effect of abrogating antisense transcription. Second, the effectiveness of the *PHO5* terminator may depend on the genomic context, and some antisense transcripts might originate from within the open reading frame of the gene, where they are not blocked by the *PHO5* terminator. Third, some antisense transcripts may have stronger effects in environments that have not yet been tested. Still, some antisense transcripts likely have little or no effect, especially those that do not overlap with the sense transcription start site. This then begs the question of what determines whether antisense transcription reaches into the sense promoter. Another interesting question is how the results obtained in yeast cells translate to organisms with a functional RNAi machinery where antisense transcripts might affect sense transcription even if there is no overlap with the sense transcriptional start site. In any case, the results of Huber et al. (2016) show that, although some antisense transcripts may simply be the result of transcriptional noise and leaky expression, many others may in fact help to control these very processes.

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